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INTERSPECIFIC DIFFERENCES IN DROSOPHILA REVEALED WITH PHOTOMETRIC ANALYSIS

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1. INTRODUCTION

The compound eye of Drosophila has been subject to considerable histological work (Nolte, 1950, 1954; Hertweck, 1931). Hadorn and collaborators (Hadorn and Mitchell, 1951; Hadorn and Kurtsteiner, 1955; Hadorn and Ziegler-Gunder, 1958) have worked on the chemical basis of the pigmentary system using mutants of Drosophila. Mainx (1938) distinguished two pigments by way of their solubility in water. Later on the work in this field was greatly simplified because the chromogens could be extracted from the heads of the flies with ethanol acidified with HCl at ph 2.0 (Ephrussi and Herold, 1944). The red and the brown pigments can also be separated during the ontogenetic formation of the pupae (Danneel, 1941). Another line of research produced results on the action of eye colour genes with the spectrophotometer (Nolte, 1952). With this tool, light extinction differences were soon found between some species (Nolte, 1958). Therefore, it appeared desirable at this point to use the pigment extraction method and the spectrophotometric measurement of the pigment in solution in order to analyse the racial affinities among members of the same species and the interspecific differences in three important species groups of the genus Drosophila.

2. MATERIALS AND METHODS

Several experiments were conducted to study the concentration of the red pigments in the eye of members of the genus Drosophila. The experiments were repeated three times, and the standard errors of the means were recorded. Although these methods should be considered satisfactory the results which they procured will be regarded as tentative. The authors hope that other laboratories will repeat these experiments in the hope that other results will correct errors and vindicate the ones here presented.

The photometric analysis of the various species were carried out with a Beckman DU spectrophotometer (tables 1 and 2) and with a Coleman photometer for the experiments on intraspecific differences within *Drosophila melanogaster* (tables 3 and 4). In the U.V spectrum a H_2 lamp was used. The visible, from 320 mu to 560 mu, was analysed with a tungsten lamp.

The red pigments were extracted with an acidified alcohol solution. Concerning the extraction method some relevant facts have to be discussed. The two differential solvents for the red and for the brown pigments are those first used by Clancy (1942). They were later utilized by Ephrussi and Herold and were named AEA and AMA. The first solvent is prepared as a 30% ethyl alcohol acidified at ph 2.0 with concentrated HC1. We confirm the stability of this solvent first realized by Clancy. Regardless of its stability, AEA was prepared every time an experiment was ready to commence.

The extraction procedure for the red pigment was as follows: the flies were decapitated with a clean razor blade at room temperature and 5 days after emergence. Four milliliters of the solvent were prepared and 20 heads without proboscis and clypeus were placed in the solvent after they dried for three minutes. No doubt this method is time consuming, however, it proved to have more merit than the other methods tried in that no turbidity formed in the extracts. Besides, our procedure permitted full extraction after 24 hrs. while the other methods recommended by the literature, require 8 or more days. The simplicity of the methods used in many laboratories consists in simply cutting off heads and placing them in the solvent. We should like to stress violent shaking during the full 24 hrs. of extraction at 22°C. All photometric readings were taken in the next 48 hrs. after extraction.

The most practical way to a quantitative determination of pigments is by measuring the light absorbed in solutions which contain the pigments. This is particularly so in the regions of the maximum absorption since there we find the most characteristic wave lengths of the substances.

The Lambert-Beer Law says that light absorption is directly proportional to the concentration of the solute and to the depth of the absorbing medium. However, since in the experiments we used cuvettes of uniform depth and kind, reason permits to ignore this variable. All our results are given in optical density units (in the figures these units are x 10^2): $E = \log I_0 / I$ with I_0 being the incident light and I the transmitted light.

Another very relevant variable is temperature. We soon found that it influences the amount of red pigments withing the species: it is well known that the relative amounts of the two red components in certain mutants vary in direct relation to temperature (Ephrussi and Herold, 1945). Besides, the total amount of pigments depend on the size of the eyes and consequently on the size of the fly. Furthermore, larvae and pupae development depends on temperature for their duration. The size of the fly is affected by the duration of its development. Therefore, it is desirable to breed all the herds, stocks and species under constant temperature: thermostatic control of incubators was necessary to maintain the temperature at 25°C. Moreover, constant size is also influenced by other culturing characteristics: the density of yeast fermentation per individual, adult crowding, density of larval population, humidity, bacterial and fungal infection (Hodson and Chiang, 1948). It was necessary to control humidity to 60% and to achieve optimum size of adults by an adequate supply of food for the larvae by introducing a sufficient amount of live Fleishman yeast suspended in water (Bakker, 1961), with a bacterial and fungal inhibitor commercially called Tegocept (Goldschmidt Co., New York). The media preparation was modified to meet our special requirements: for every 1.000 cc. of water, 35 grams of Agar-agar, 16 bananas and 20 cc. of Tegocept. The Tegocept was a 10% solution of 75% ethyl alcohol. Every half pound of medium contained 1 cc. of Fleishman yeast which was added 10 hrs. after the culture medium was freshly prepared, and 24 hrs. before the individuals were introduced in order to have fermentation started.

To have cultures with 200 larvae, 5 females freshly brought from nature were placed with 3 males from the same locality, properly labelled, for 48 hrs. to produce 250 eggs during that time. The oviposition is approximately constant and uniform if males and females are placed on fresh media for the first 6 days of imaginal life in the laboratory. Populations developing under these conditions do not exceed 180 individuals per culture. After six generations under such conditions body size and consequently eye size show little variation.

Individuals resulting from such cultures were used for the extraction of pigment. Removal of eggs due to excessive laying was necessary in some cases. Usually, however, the number of flies varied from 150 to 180 per culture medium.

3. RESULTS AND DISCUSSION

Brevity advises us to recommend the reader direct examination of tables and figures. Each species presents its own characteristic spectrograph, although there are intraspecific fluctuations. In some cases the differences are present in the U.V part of the spectrum only (females of *D. melanogaster* and of *D. ananassae*); while in other cases the differences are evident in the visible spectrum as well as in the U. V. Interspecific similarities coincide with philogenetic affinities, while racial similarities are indicative of the common genetic pool all these populations share.

The authors call attention to the widely different habitats from which the races of *D. melanogaster* came. What affected the survival and the reproduction of the individuals from the low lands should be totally different in intensity, at least, from the circumstances that affected the individuals living in the caribbean island of San Andrés: therefore, the two radically different environments have almost certainly elicited different genetic structures (Dobzhansky, 1951). We are defining environment as the sum of circumstances that may influence a population in its survival and in its multiplication (Andrewartha, 1963). In the South American continent a) weather (light, humidity, pressure, temperature) b) food c) predators d) pathogens and e) peculiar conditions of the terrain are different from the semi-desertic hot northern coast (Soledad, Caracolicito and Santa Marta) to the Sinú valley (Montería: hot humid, dense tropical forest) that adaptive peaks, genetic-wise, must have produced in time different genotypes within the same Mendelian pool. *D. melanogaster*, *D. ananassae*, *D. pseudoobscura*, *D. repleta*, *D. nebulosa* and *D. willistoni* respond so differently in eye pigmentation to these same environments that their photometric differences are much greater than those between members of the same species. The racial dissimilarities are many time smaller than the interspecific ones. At the same time there are species which differ less: *melanogaster* and *ananassae* on one hand, and *nebulosa* and *willistoni* on the other hand. These similarities at the interspecific level reveal their reputed philogenetic kinship.

4. SUMMARY

The above refer to experiments present a new method which permits the study of philogenesis in the genus Drosophila. There are several types of results: a) close kinship among the various geographical races of *D. melanogaster* in the neo-tropics coincides with their spectrophotometric similarities; b) the interspecific differences are also identified with the photometric analysis; c) finally there are optical density affinities among the various species which belong to the same taxonomic groups.

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TABLE 1. Optical densities of the U. V. spectrum and standard errors of the means of 3 replicas taken from red pigments extracted by the method explained in the text. These data correspond to females of the following species: 1) *D. pseudoobscura*; 2) *D. willistoni*; 3) *D. nebulosa*; 4) *D. melanogaster*, and 5) *D. ananassae.*

Optical densities and standard errors.

λ	1	2	3	4	5
220	0.838 ± 0.0003	1.080 ± 0.0001	1.130 ± 0.0001	0.540 ± 0.0001	0.770 ± 0.0001
225	0.742 ± 0.0001	1.020 ± 0.0001	1.020 ± 0.0001	0.512 ± 0.0001	0.690 ± 0.0001
230	0.588 ± 0.0001	0.830 ± 0.0038	0.840 ± 0.0005	0.394 ± 0.0005	0.580 ± 0.0001
235	0.449 ± 0.0001	0.668 ± 0.0001	0.651 ± 0.0001	0.301 ± 0.0003	0.440 ± 0.0001
240	0.385 ± 0.0001	0.580 ± 0.0001	0.565 ± 0.0003	0.250 ± 0.0001	0.372 ± 0.0001
245	0.373 ± 0.0015	0.539 ± 0.0005	0.507 ± 0.0001	0.242 ± 0.0001	0.345 ± 0.0005
250	0.366 ± 0.0006	0.518 ± 0.0001	0.475 ± 0.0001	0.226 ± 0.0005	0.323 ± 0.0001
255	0.354 ± 0.0003	0.488 ± 0.0001	0.432 ± 0.0001	0.228 ± 0.0010	0.313 ± 0.0003
260	0.347 ± 0.0001	0.480 ± 0.0001	0.431 ± 0.0003	0.227 ± 0.0003	0.312 ± 0.0003
265	0.360 ± 0.0003	0.509 ± 0.0011	0.472 ± 0.0003	0.248 ± 0.0001	0.330 ± 0.0001
270	0.373 ± 0.0005	0.558 ± 0.0001	0.531 ± 0.0001	0.278 ± 0.0003	0.352 ± 0.0001
275	0.382 ± 0.0003	0.600 ± 0.0001	0.589 ± 0.0001	0.277 ± 0.0001	0.368 ± 0.0001
280	0.377 ± 0.0001	0.617 ± 0.0001	0.610 ± 0.0001	0.277 ± 0.0003	0.370 ± 0.0001
285	0.343 ± 0.0013	0.579 ± 0.0001	0.588 ± 0.0001	0.257 ± 0.0001	0.352 ± 0.0001
290	0.295 ± 0.0003	0.505 ± 0.0001	0.511 ± 0.0004	0.224 ± 0.0004	0.304 ± 0.0003
295	0.238 ± 0.0005	0.401 ± 0.0003	0.400 ± 0.0001	0.178 ± 0.0006	0.247 ± 0.0001
300	0.184 ± 0.0001	0.285 ± 0.0009	0.282 ± 0.0008	0.132 ± 0.0001	0.187 ± 0.0001
305	0.143 ± 0.0001	0.207 ± 0.0001	0.188 ± 0.0001	0.100 ± 0.0001	0.140 ± 0.0003
310	0.124 ± 0.0001	0.160 ± 0.0008	0.139 ± 0.0017	0.084 ± 0.0001	0.116 ± 0.0005
315	0.118 ± 0.0001	0.145 ± 0.0001	0.124 ± 0.0005	0.077 ± 0.0006	0.107 ± 0.0001
320	0.118 ± 0.0001	0.147 ± 0.0001	0.125 ± 0.0001	0.078 ± 0.0005	0.107 ± 0.0005

TABLE 2. Optical densities of the visible spectrum and standard errors of the means of 3 replicas taken from red pigments extracted by the method explained in the text. These data are taken from females of the following species: 1) *D. pseudoobscura*; 2) *D. willistoni*; 3) *D. nebulosa*; 4) *D. melanogaster*; 5) *D. ananassae*, and 6) *D. prosaltans.*

Optical densities and standard errors.

λ	1	2	3	4	5	6
330	0.083 ± 0.0001	0.125 ± 0.0001	0.092 ± 0.0003	0.058 ± 0.0003	0.080 ± 0.0005	
340	0.080 ± 0.0001	0.107 ± 0.0001	0.083 ± 0.0005	0.054 ± 0.0001	0.080 ± 0.0001	
350	0.064 ± 0.0005	0.089 ± 0.0005	0.069 ± 0.0003	0.042 ± 0.0001	0.060 ± 0.0001	
360	0.062 ± 0.0001	0.079 ± 0.0005	0.061 ± 0.0003	0.038 ± 0.0001	0.052 ± 0.0006	
370	0.062 ± 0.0001	0.076 ± 0.0001	0.053 ± 0.0001	0.041 ± 0.0005	0.050 ± 0.0001	
380	0.050 ± 0.0001	0.075 ± 0.0001	0.054 ± 0.0003	0.040 ± 0.0001	0.050 ± 0.0001	
390	0.065 ± 0.0001	0.079 ± 0.0001	0.065 ± 0.0003	0.047 ± 0.0001	0.060 ± 0.0011	
400	0.082 ± 0.0001	0.108 ± 0.0013	0.087 ± 0.0001	0.064 ± 0.0005	0.077 ± 0.0001	0.094 ± 0.0004
420	0.147 ± 0.0003	0.205 ± 0.0001	0.175 ± 0.0001	0.119 ± 0.0013	0.142 ± 0.0005	0.148 ± 0.0008
440	0.243 ± 0.0001	0.342 ± 0.0001	0.294 ± 0.0003	0.194 ± 0.0001	0.264 ± 0.0024	0.238 ± 0.0005
460	0.343 ± 0.0001	0.472 ± 0.0001	0.409 ± 0.0003	0.267 ± 0.0001	0.280 ± 0.0005	0.338 ± 0.0005
470	0.364 ± 0.0001	0.500 ± 0.0001	0.431 ± 0.0006	0.284 ± 0.0001	0.300 ± 0.0001	0.373 ± 0.0008
480	0.362 ± 0.0001	0.503 ± 0.0008	0.438 ± 0.0001	0.290 ± 0.0001	0.305 ± 0.0008	0.400 ± 0.0001
490	0.337 ± 0.0001	0.477 ± 0.0016	0.411 ± 0.0001	0.274 ± 0.0001	0.288 ± 0.0001	0.400 ± 0.0001
500	0.294 ± 0.0001	0.427 ± 0.0001	0.354 ± 0.0001	0.243 ± 0.0001	0.252 ± 0.0014	0.378 ± 0.0001
520	0.137 ± 0.0001	0.186 ± 0.0005	0.150 ± 0.0003	0.106 ± 0.0004	0.115 ± 0.0003	0.226 ± 0.0013
540	0.035 ± 0.0001	0.050 ± 0.0001	0.036 ± 0.0001	0.027 ± 0.0001	0.030 ± 0.0001	0.071 ± 0.0005
560	0.007 ± 0.0001	0.017 ± 0.0001	0.007 ± 0.0004	0.005 ± 0.0001	0.006 ± 0.0001	0.019 ± 0.0001

TABLE 3. Optical densities of the visible spectrum and standard errors of the means of 3 replicas taken from red pigments extracted by the method explained in the text. These data correspond to males from the following habitats of *D. melanogaster*: 1) Caracolicito; 2) Montería; 3) S. Andrés; 4) Medellín; 5) Bogotá; 6) S. Jorge; 7) Duitama, and 8) S. Marta.

Optical densities and standard errors.

λ	1	2	3	4	5	6	7	8
400	0.063 ± 0.0008	0.052 ± 0.0015	0.035 ± 0.0001	0.038 ± 0.0003	0.043 ± 0.0017	0.056 ± 0.0008	0.044 ± 0.0008	0.052 ± 0.0001
420	0.102 ± 0.0007	0.080 ± 0.0017	0.066 ± 0.0008	0.070 ± 0.0001	0.071 ± 0.0003	0.085 ± 0.0001	0.075 ± 0.0001	0.088 ± 0.0001
440	0.177 ± 0.0020	0.134 ± 0.0016	0.105 ± 0.0001	0.110 ± 0.0001	0.120 ± 0.0001	0.138 ± 0.0005	0.118 ± 0.0001	0.145 ± 0.0001
445	0.198 ± 0.0004	0.152 ± 0.0001	0.121 ± 0.0013	0.127 ± 0.0011	0.133 ± 0.0008	0.150 ± 0.0003	0.131 ± 0.0003	0.161 ± 0.0003
450	0.251 ± 0.0015	0.163 ± 0.0005	0.132 ± 0.0001	0.137 ± 0.0008	0.149 ± 0.0004	0.162 ± 0.0001	0.144 ± 0.0001	0.180 ± 0.0001
455	0.232 ± 0.0014	0.179 ± 0.0004	0.148 ± 0.0001	0.148 ± 0.0001	0.160 ± 0.0005	0.117 ± 0.0005	0.160 ± 0.0001	0.193 ± 0.0013
460	0.252 ± 0.0012	0.196 ± 0.0012	0.158 ± 0.0001	0.161 ± 0.0005	0.173 ± 0.0005	0.193 ± 0.0008	0.170 ± 0.0001	0.210 ± 0.0001
465	0.268 ± 0.0008	0.208 ± 0.0005	0.167 ± 0.0019	0.171 ± 0.0013	0.181 ± 0.0004	0.201 ± 0.0012	0.178 ± 0.0005	0.220 ± 0.0001
470	0.282 ± 0.0010	0.215 ± 0.0003	0.175 ± 0.0001	0.182 ± 0.0012	0.193 ± 0.0005	0.211 ± 0.0001	0.185 ± 0.0001	0.232 ± 0.0001
475	0.292 ± 0.0011	0.225 ± 0.0014	0.182 ± 0.0001	0.188 ± 0.0001	0.200 ± 0.0001	0.221 ± 0.0005	0.199 ± 0.0005	0.240 ± 0.0003
480	0.298 ± 0.0005	0.229 ± 0.0010	0.190 ± 0.0001	0.191 ± 0.0013	0.202 ± 0.0011	0.222 ± 0.0001	0.200 ± 0.0001	0.248 ± 0.0001
485	0.300 ± 0.0009	0.229 ± 0.0007	0.190 ± 0.0001	0.190 ± 0.0003	0.206 ± 0.0008	0.229 ± 0.0007	0.200 ± 0.0005	0.247 ± 0.0008
490	0.299 ± 0.0007	0.230 ± 0.0003	0.190 ± 0.0001	0.190 ± 0.0003	0.205 ± 0.0001	0.226 ± 0.0008	0.198 ± 0.0005	0.247 ± 0.0008
495	0.291 ± 0.0004	0.225 ± 0.0001	0.185 ± 0.0001	0.187 ± 0.0008	0.204 ± 0.0008	0.220 ± 0.0001	0.196 ± 0.0008	0.243 ± 0.0008
500	0.280 ± 0.0004	0.212 ± 0.0001	0.178 ± 0.0001	0.180 ± 0.0001	0.192 ± 0.0001	0.210 ± 0.0001	0.188 ± 0.0001	0.233 ± 0.0008
505	0.258 ± 0.0008	0.196 ± 0.0008	0.165 ± 0.0001	0.168 ± 0.0003	0.178 ± 0.0001	0.197 ± 0.0001	0.174 ± 0.0003	0.215 ± 0.0001
510	0.231 ± 0.0005	0.178 ± 0.0008	0.150 ± 0.0001	0.152 ± 0.0001	0.160 ± 0.0001	0.175 ± 0.0001	0.158 ± 0.0001	0.196 ± 0.0004
515	0.198 ± 0.0007	0.152 ± 0.0008	0.128 ± 0.0001	0.130 ± 0.0003	0.138 ± 0.0005	0.155 ± 0.0005	0.138 ± 0.0005	0.167 ± 0.0003
520	0.163 ± 0.0018	0.125 ± 0.0001	0.108 ± 0.0001	0.110 ± 0.0001	0.115 ± 0.0014	0.128 ± 0.0001	0.116 ± 0.0005	0.138 ± 0.0001
540	0.052 ± 0.0001	0.037 ± 0.0008	0.034 ± 0.0008	0.033 ± 0.0008	0.036 ± 0.0007	0.040 ± 0.0009	0.040 ± 0.0009	0.047 ± 0.0001
560	0.103 ± 0.0003	0.008 ± 0.0001	0.008 ± 0.0001	0.012 ± 0.0001	0.007 ± 0.0012	0.014 ± 0.0003	0.008 ± 0.0003	0.011 ± 0.0005

TABLE 4. Optical densities of the visible spectrum and standard errors of the means of 3 replicas taken from red pigments extracted by the method explained in the text. These data correspond to females from the following habitats of *D. melanogaster*: 1) Caracolicito; 2) Montería; 3) S. Marta; 4) S. Andrés; 5) Medellín; 6) Bogotá; 7) S. Jorge, and 8) Soledad.

Optical densities and standard errors.

λ	1	2	3	4	5	6	7	8
400	0.066 ± 0.0013	0.036 ± 0.0005	0.044 ± 0.0024	0.040 ± 0.0003	0.051 ± 0.0008	0.046 ± 0.0006	0.050 ± 0.0001	0.050 ± 0.0003
420	0.122 ± 0.0007	0.070 ± 0.0008	0.072 ± 0.0001	0.068 ± 0.0001	0.076 ± 0.0005	0.070 ± 0.0008	0.074 ± 0.0003	0.080 ± 0.0001
440	0.210 ± 0.0001	0.124 ± 0.0008	0.118 ± 0.0003	0.114 ± 0.0004	0.130 ± 0.0001	0.128 ± 0.0001	0.128 ± 0.0003	0.132 ± 0.0003
445	0.236 ± 0.0019	0.139 ± 0.0011	0.131 ± 0.0004	0.133 ± 0.0008	0.144 ± 0.0008	0.133 ± 0.0008	0.139 ± 0.0003	0.145 ± 0.0001
450	0.259 ± 0.0001	0.155 ± 0.0004	0.114 ± 0.0012	0.141 ± 0.0003	0.155 ± 0.0021	0.145 ± 0.0003	0.150 ± 0.0005	0.160 ± 0.0001
455	0.286 ± 0.0008	0.174 ± 0.0004	0.157 ± 0.0025	0.155 ± 0.0001	0.170 ± 0.0011	0.158 ± 0.0001	0.166 ± 0.0005	0.177 ± 0.0005
460	0.310 ± 0.0004	0.187 ± 0.0007	0.170 ± 0.0028	0.168 ± 0.0003	0.185 ± 0.0001	0.166 ± 0.0013	0.180 ± 0.0001	0.190 ± 0.0003
465	0.336 ± 0.0016	0.206 ± 0.0019	0.178 ± 0.0019	0.178 ± 0.0003	0.197 ± 0.0012	0.180 ± 0.0005	0.185 ± 0.0001	0.200 ± 0.0001
470	0.352 ± 0.0003	0.213 ± 0.0008	0.190 ± 0.0004	0.189 ± 0.0004	0.204 ± 0.0008	0.190 ± 0.0008	0.196 ± 0.0013	0.207 ± 0.0011
475	0.365 ± 0.0005	0.223 ± 0.0008	0.198 ± 0.0003	0.190 ± 0.0003	0.214 ± 0.0003	0.200 ± 0.0001	0.208 ± 0.0001	0.219 ± 0.0005
480	0.372 ± 0.0004	0.227 ± 0.0010	0.199 ± 0.0005	0.199 ± 0.0005	0.216 ± 0.0008	0.200 ± 0.0001	0.210 ± 0.0001	0.222 ± 0.0001
485	0.375 ± 0.0003	0.229 ± 0.0005	0.201 ± 0.0008	0.201 ± 0.0001	0.220 ± 0.0001	0.200 ± 0.0001	0.210 ± 0.0001	0.220 ± 0.0005
490	0.374 ± 0.0003	0.227 ± 0.0005	0.200 ± 0.0005	0.199 ± 0.0002	0.220 ± 0.0001	0.199 ± 0.0003	0.210 ± 0.0001	0.221 ± 0.0001
495	0.369 ± 0.0005	0.226 ± 0.0004	0.194 ± 0.0009	0.198 ± 0.0001	0.215 ± 0.0001	0.198 ± 0.0003	0.210 ± 0.0001	0.221 ± 0.0003
500	0.354 ± 0.0002	0.215 ± 0.0004	0.187 ± 0.0008	0.190 ± 0.0001	0.205 ± 0.0010	0.190 ± 0.0001	0.197 ± 0.0002	0.213 ± 0.0003
505	0.329 ± 0.0014	0.203 ± 0.0011	0.171 ± 0.0003	0.175 ± 0.0001	0.190 ± 0.0005	0.176 ± 0.0001	0.184 ± 0.0003	0.199 ± 0.0005
510	0.300 ± 0.0003	0.183 ± 0.0003	0.155 ± 0.0004	0.158 ± 0.0001	0.172 ± 0.0001	0.156 ± 0.0005	0.165 ± 0.0001	0.175 ± 0.0001
515	0.257 ± 0.0012	0.159 ± 0.0012	0.131 ± 0.0003	0.138 ± 0.0003	0.148 ± 0.0008	0.137 ± 0.0008	0.140 ± 0.0005	0.151 ± 0.0003
520	0.214 ± 0.0008	0.134 ± 0.0010	0.101 ± 0.0005	0.112 ± 0.0001	0.120 ± 0.0001	0.111 ± 0.0013	0.119 ± 0.0005	0.127 ± 0.0008
540	0.066 ± 0.0008	0.040 ± 0.0001	0.032 ± 0.0010	0.040 ± 0.0001	0.034 ± 0.0008	0.038 ± 0.0001	0.036 ± 0.0013	0.040 ± 0.0005
560	0.024 ± 0.0016	0.014 ± 0.0008	0.006 ± 0.0011	0.008 ± 0.0001	0.008 ± 0.0001	0.012 ± 0.0001	0.009 ± 0.0005	0.010 ± 0.0001

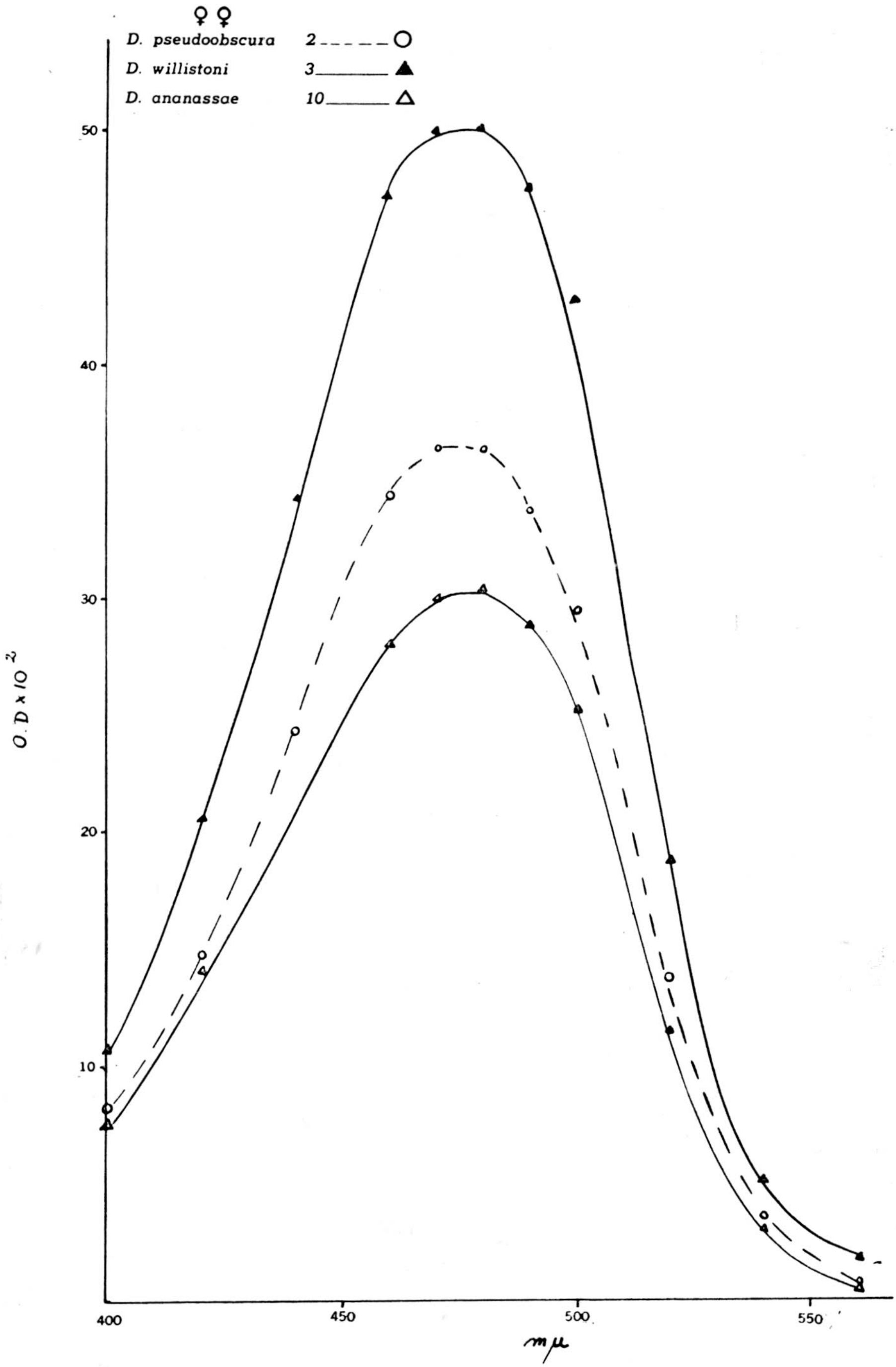

Figure 1. For explanations see the text.

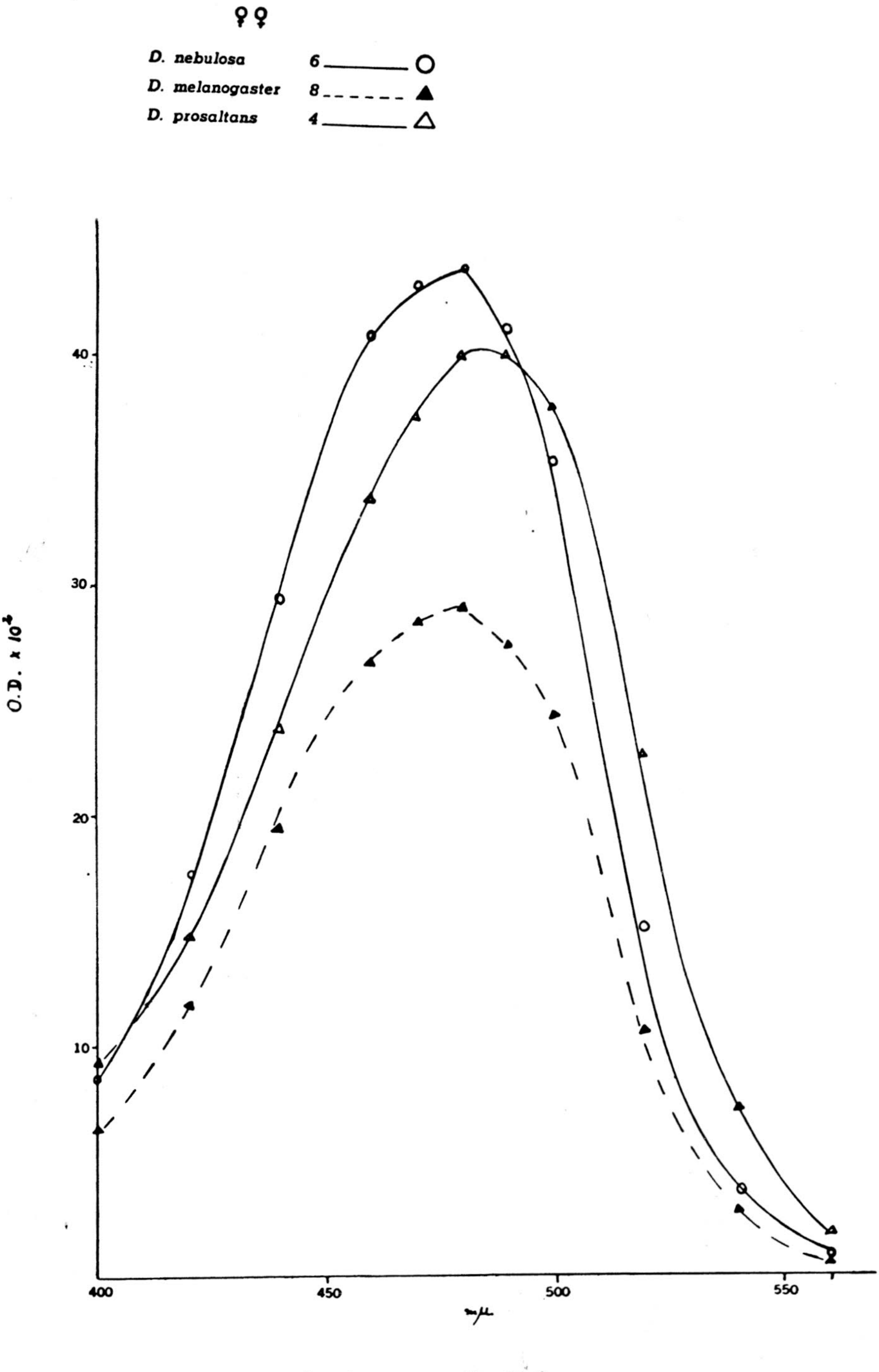

Figure 2. For explanations see the Text.

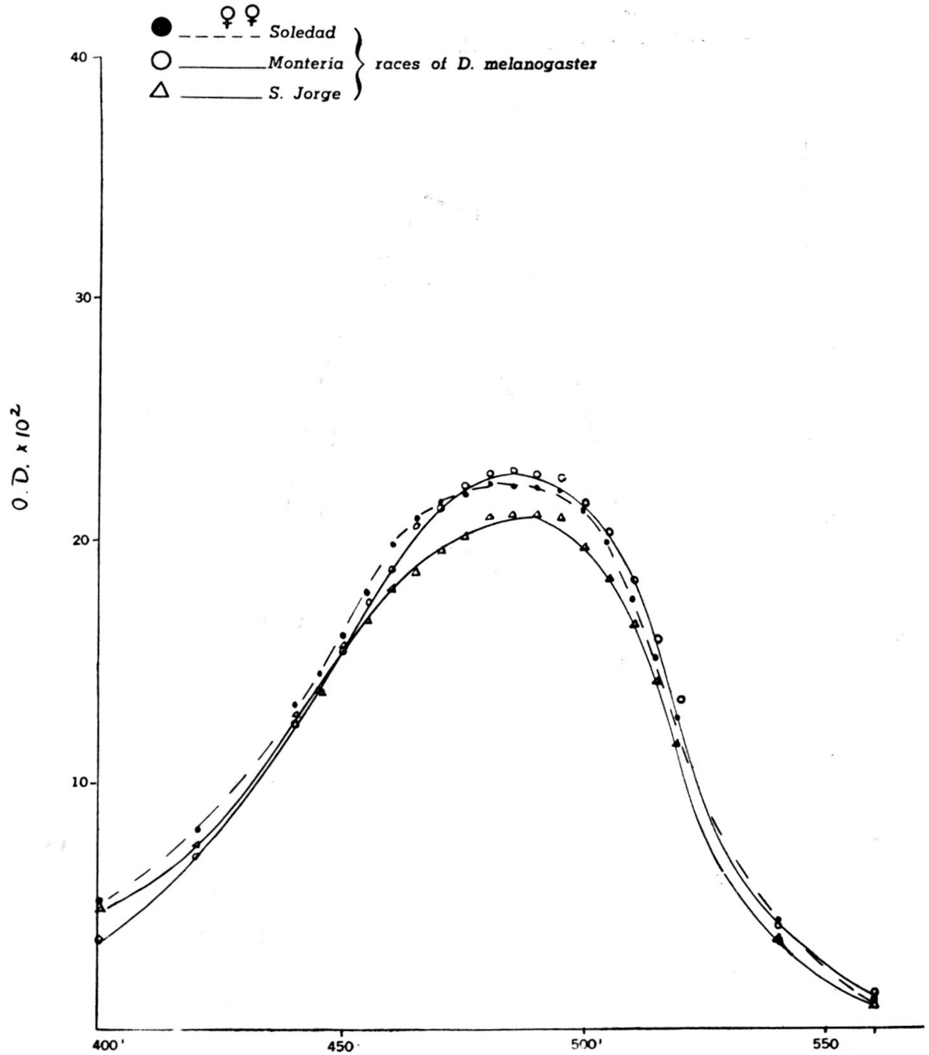

Figure 3. For explanations see the text.

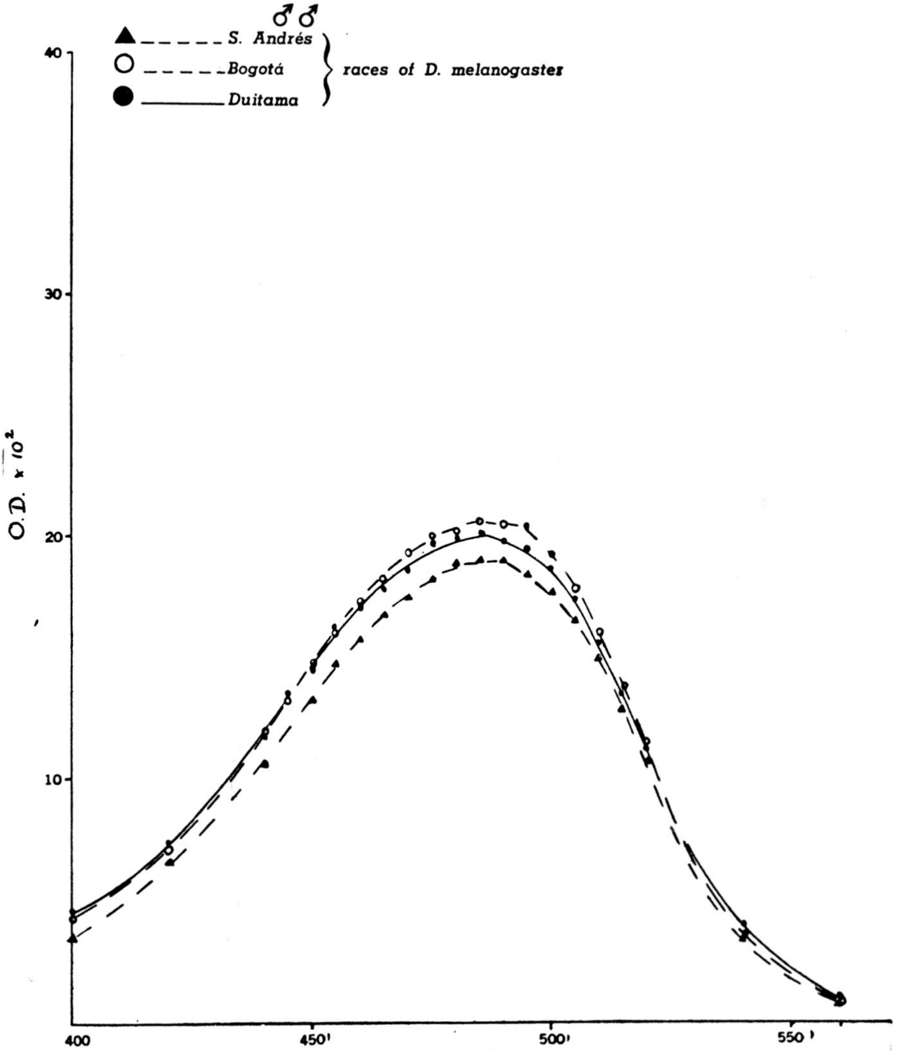

Figure 4. For explanations see the text.